Intracellular (polymorphonuclear) magnesium content in patients with bronchial asthma between attacks

Panayotis Fantidis MD PhD[1] Jesús Ruiz Cacho MD[2] Mercedes Marín MD[3] Rosario Madero Jarabo[4] Jesús Solera MD PhD[2] Elisa Herrero MD[2]

J R Soc Med 1995;**88**:441–445

Keywords: *magnesium; polymorphonuclear; asthmatics*

Previous studies have demonstrated the role of polymorphonuclears in asthma, the importance of increases in intracellular concentrations of ionized calcium in the release of chemical mediators, the role of magnesium as natural calcium channel blocker, and the importance of magnesium deficiency in plasma histamine concentration and in tissue prostanoid concentration. The objective of our study was to measure the polymorphonuclear magnesium content in patients with asthma in the interval between attacks.

We measured the polymorphonuclear magnesium content, and serum and erythrocyte sodium, calcium, and magnesium concentrations in 21 healthy volunteers and 50 patients with different types of asthma. In our patients, the polymorphonuclear magnesium content was lower than in the control group ($P<0.001$), while magnesium levels in erythrocytes and serum and the levels of other ions in erythrocytes and serum were normal.

This is the first report to document low polymorphonuclear magnesium content in patients with bronchial asthma. The reduction of polymorphonuclear magnesium content may have an important role in the pathogenesis of asthma.

INTRODUCTION

In recent years, several authors have reported obtaining beneficial effects with magnesium therapy in different types of asthma, including the inhibition of bronchoconstriction in asthmatic patients challenged with a methacholine or histamine bronchoprovocation test[1–6]. It has been postulated that the therapeutic effect of magnesium in asthma derives from its action in modulation of smooth muscle contractility[1–7] and in mediator release through its antagonism of the action of calcium at any one of several sites[8,9].

Basophils and mast cells are target cells for IgE molecules. In asthma, these cells release chemical mediators on appropriate antigen challenge[10,11]. The primary trigger for the release of chemical mediators from basophils and mast cells is a rise in cellular Ca^{++} concentration[12]. As magnesium is a natural calcium channel blocker[8,9,13], a deficiency in polymorphonuclear magnesium content could potentiate the action of Ca^{++}.

Mastocytes are the most important source of prostaglandin D_2, which exercises synergistic effects with other eicosanoids causing contractions of airway smooth muscle[14]. It has been demonstrated that the action of prostanoids on smooth muscle is magnesium-dependent, and tissue levels of prostanoids are higher in magnesium-deficient rats[15]. Increased synthesis of prostanoids is linked to enhanced cellular influx and translocation of Ca^{++}[16]. Magnesium deficiency in rats has been shown to produce an increase in urinary and plasma histamine levels[17].

Hence, a possible reduction of polymorphonuclear magnesium content (plMg) may have an important role in the pathogenesis of asthma. In this clinical study, we measured polymorphonuclear cell magnesium content, erythrocyte sodium, potassium, and magnesium content, and serum calcium, potassium, sodium, and magnesium concentrations in 21 healthy volunteers and 50 patients with bronchial asthma in the interval between attacks.

MATERIAL AND METHODS

We studied a total of 71 adults of both sexes, ranging in age from 25 to 55 years. Any patient or subject in the control group who had a cause of potential Mg deficit (i.e. pregnancy, breastfeeding, recent convalescence, menopause, menstrual disorders which require treatment, men over 55 years, kidney disease, bone disease, gastrointestinal disease, cardiovascular disease, hormonal disorders, metabolic disorders, recent infections), or signs suggestive of potential Mg deficit (i.e. frequent cramps, frequent palpitations, spasms, asthenia, anorexia, dysphagia, tremor, excessive physical stress, anxiety), was excluded as inclusion

[1]Departments of Research Unit—Experimental Surgery, [2]Biochemistry, [3]Respiratory Rehabilitation Services and [4]Biostatistics, Hospital 'La Paz', School of Medicine, Universidad Autónoma, Madrid, Spain

Correspondence to: Dr Panayotis Fantidis, c/ Santa Virgilia 11, Esc. izq 3°B, 28033 Madrid, Spain

of these subjects would have confused the issue of whether deficient polymorphonuclear magnesium content is inherent to asthma. Other reasons for exclusion were current or recent (up to 1 month before study) treatment with contraceptives, calcium antagonists, diuretics with the exception of potassium-sparing agents, digoxin, laxatives, antibiotics, vitamin D, or abusive consumption of alcohol, salt or salted products, food additives, prepared foods, animal viscera, sugar, animal fat, milk and milk-products. No asthma attack for at least 1 week before the study was another necessary criteria for inclusion. The subjects in the control group and patients in the study group were duly informed, and all gave their written consent, which was approved by the Human Studies Committee of 'La Paz' Hospital. Blood samples were taken from the persons in the control group on three different days: patients' blood samples were obtained on days when routine analytic follow-ups were scheduled.

Twenty-one volunteers, all employees of our hospital, were selected for the control group. All were healthy and had neither allergy to medications, foods, or other agents, nor episodes of allergic rhinitis or asthma. Asthma was diagnosed on the basis of clinical diagnosis (history and physical examination), associated signs of allergy, laboratory diagnosis (complete blood count, sputum and nasal mucus cytology), radiography and lung function tests. The study group contained: 50 asthmatic patients; 16 with mild bronchial asthma; 22 moderate asthma; 12 severe asthma. Patients with mild asthma who had only intermittent asthma attacks, were completely free of symptoms in the period between attacks, and who had been managed with aerosolized adrenergic solutions were included. The asthma in patients with moderate asthma interfered with the individual's usual activities and patients had abnormal pulmonary function (FVC, FEV_1, FEV_1/FVC and MEF; reduced, between 20–30%). Patients with severe asthma had incapacitating dyspnea, cough, and obstructed airways in spite of treatment and persistently abnormal pulmonary function (FVC, FEV_1, FEV_1/FVC, and MEF; reduced more than 30%). Patients with moderate asthma were daily receiving aerosolized solutions (β-2 stimulants and corticosteroids), and oral medication with theophylline. Patients with severe asthma were receiving nebulized solutions and oral medication with theophylline and corticosteroid. Thirty-three patients had intrinsic asthma and 17 had extrinsic asthma.

In all 71 study subjects, serum sodium and potassium concentrations were measured with an ion-selective electrode (Synchron El-ise, Electrolyte System, Beckman, USA), and serum calcium and magnesium concentrations were assayed by means of atomic absorption spectrophotometry (Perkin Elmer model 460 atomic absorption spectrophotometer). After lysing erythrocytes in a volume of distilled deionized water equal to that of the packed erythrocytes, erythrocyte sodium and potassium concentrations were measured by flame photometry (Instrumentation Laboratory 943 flame photometer), and erythrocyte magnesium concentration was measured with atomic absorption spectrophotometry (Perkin Elmer model 460 atomic absorption spectrophotometer).

The magnesium content of polymorphonuclear cells was measured using techniques described previously[18–20]. Platelets were eliminated from whole blood and leukocytes were separated by density gradient centrifugation and counted. Magnesium and DNA concentrations were measured in the cellular lysate. Platelets were eliminated from 6 ml of whole blood anticoagulated with heparin by centrifuging at 250 G for 15 min. The platelet-rich plasma was removed and centrifuged separately, after which the platelet-free plasma was returned to the sample. This procedure was repeated twice to ensure the total elimination of platelets. After the last centrifugation, the supernatant was replaced by an equal volume of phosphate buffer saline. To separate the leukocytes, 3 ml of histopaque 1119, 3 ml of histopaque 1077, and 6 ml of platelet-free whole blood was deposited in a centrifuge tube and centrifuged at 700 G for 30 min at room temperature. Two separate bands corresponding to the interphases were observed: an upper band of mononuclear cells; and a lower band of granulocytes. The two cellular bands were aspirated and transferred to different tubes, washed three times with phosphate buffer saline, then centrifuged at 200 G for 10 min. The resulting pellet was resuspended in an appropriate volume of phosphate buffer saline. The pellet of polymorphonuclear cells was lysed ultrasonically for 180 sec, and the magnesium in the cellular lysate was measured with flame absorption spectrophotometry (Perkin Elmer model 460 spectrophotometer). DNA was measured by means of fluorometry according to a technique described elsewhere[20]. Polymorphonuclear cell magnesium content was expressed as mg MG/ng DNA.

Statistical analysis was carried out with a set of BMDP programs running on a PS model 50-Z computer. The Student t test was used as a parametic test and the Wilcoxon test as a non-parametric test. $P<0.05$ was considered significant. Stepwise discriminant analysis was performed.

RESULTS

In the control group of 21 volunteers (Table 1), polymorphonuclear magnesium content was 1.84 ± 0.97 SD mg Mg/ng DNA (median; 1.3 mg Mg/ng DNA, coefficient interval for median—CIM; 1–2.6 mg Mg/ng DNA). The overall polymorphonuclear magnesium content in the patient group was 0.88 (standard

Table 1 **Laboratory values**

	Control (n=21)	*Asthmatic (n=50)*	*Low-plMg (n=34)*	*N-plMg (n=16)*
plMg content	1.84±0.9	0.88±0.61*	0.38±0.28*	1.88±0.9
Na Erythrocyte	19.3±4.4	20.7±9.3	20.6±9	20.8±9.8
K Erythrocyte	38±4.5	39.7±6.3	39±6.1	41±6.6
Mg Erythrocyte	5.6±0.7	5.2±0.6	5.2±0.	5.3±0.5
Ca Serum	2.35±0.18	2.34±0.11	2.34±0.09	2.24±0.07
K Serum	4.3±0.3	4.3±0.2	4.3±0.2	4.2±0.3
Na Serum	140.8±3.6	139±2.4	139.8±2.4	141±3.2
Mg Serum	0.77±0.06	0.77±0.06	0.77±0.05	0.781±0.04

P: Values statistically significant versus control values. Values given as mean±SD
plMg=Plymorphonuclear magnesium content, mg Mg/ng DNA; Asthmatics=patients with bronchial asthma; Low-plMg=subgroup of patients with low plMg content; N-plMg=subgroup of patients with normal plMg content. Erythrocyte Na, K and Mg concentrations are given as: mg/l cells

direction—MD—mg Mg/ng DNA; median 0.64 mg Mg/ng DNA, CIM 0.35–1.15 mg Mg/ng DNA). The difference in polymorphonuclear magnesium content between this group and the control group being statistically significant ($P<0.001$, parametric and non-parametric tests). Of the group of asthmatics, one subgroup of 34 patients had a polymorphonuclear magnesium content of 0.38±0.28 mg Mg/ng DNA (range 0.033–0.83 mg Mg/ng DNA: median 0.3 mg Mg/ng DNA, CIM; 0.05–0.65 mg Mg/ng DNA). The difference in plMg content between this subgroup of patients and the control group being statistically significant ($P<0.0001$, parametric and non-parametric tests). The remaining 16 asthmatic patients had a polymorphonuclear magnesium content of 1.88±0.99 mg Mg/ng DNA (range 1–2.79 mg Mg/ng DNA: median 1.5 mg Mg/ng DNA, CIM: 1.2–2 mg Mg/ng DNA). The difference in comparison with the control group not being statistically significant (Table 1).

Table 1 also gives means and SD of serum and erythrocyte ion concentrations in normal controls and asthmatics. There were no statistically significant differences in mean ion concentrations between the group of asthmatic patients and the control group.

Stepwise discriminant analysis showed that polymorphonuclear magnesium content discriminated between the control group and group of asthmatics, and between the control group and low-plMg subgroup of asthmatics. No variable discriminated between the control group and the normal-plMg subgroup of asthmatics.

DISCUSSION

Our study excluded all subjects with any signs of disorders other than asthma that might affect magnesium concentration. Although the exclusion criteria of 'abusive consumption' are subjective, they were adopted because of the difficulty of knowing the exact amount of every product that each patient had consumed per day, week, or month, and, above all, because of the impossibility of knowing how each product influences magnesium levels and in what amounts they do not affect magnesium levels. Thus, any variation in the magnesium content of polymorphonuclear cells in the patients with bronchial asthma could be considered as inherent to bronchial asthma.

Patients with either intrinsic or extrinsic asthma were included in our study because: (1) enhanced basophilic histamine release occurs in both types of asthma[21,22]: (2) intracellular magnesium levels is the best indicator of the whole-body magnesium content[23,24]. As polymorphonuclear cells play an important role in asthma[10,11,25], we measured polymorphonuclear magnesium content (plMg). In our 50 patients, polymorphonuclear magnesium content was lower than in the control group (Table 1). Stepwise discriminant analysis showed that plMg content discriminated between the control group and group of asthmatics.

It has been demonstrated that a higher intracellular concentration of adenosine 3′,5′ cyclic monophosphate (cAMP) in basophils inhibits mediator activation secretion[26], and increased cyclic guanosine monophosphate levels tend to increase chemical mediator release[25]. Adenylate cyclase which is a membrane-bound, magnesium-dependent enzyme; is inhibited by calcium ions[25,27] and calcium ions stimulate quanylate cyclase[25]. It has been found that unstimulated cells from asthmatic subjects have a lower cAMP concentration[28].

Prostaglandin D_2 and leukotrienes have been shown to play an important role in asthma. They cause potent bronchoconstriction, and increase mucus secretion[14,15,29–35]. The biosynthesis of prostaglandins and leukotrienes is dependent on the availability of free arachidonic acid derived from membrane phospholipids. Phosphorylase A_2 the most probable enzyme for regulating arachidonic acid production is a membrane-bound, Ca^{++} dependent enzyme[36]. Prostaglandins and leukotrienes are formed by

the oxygenation of arachidonic acid by cyclooxygenase and lipoxygenase, respectively. Both are Ca^{++} dependent enzymes[37]. It has been demonstrated that small increases in intracellular calcium promote the secretion of only cyclooxygenase products, while larger increases in intracellular calcium cause secretion of both cyclooxygenase and lipoxygenase products[38].

From the above, it can be deduced that the polymorphonuclear magnesium deficiency found in our patients with bronchial asthma between attacks could potentiate the action of Ca^{++}, reducing the intracellular levels of cAMP and increasing synthesis of leukotrienes and prostaglandins. In view of the fact that Mg levels in erythrocytes and serum were normal and that the levels of other ions in erythrocytes and serum were also normal, the reason for the low-plMg content of this subgroup of patients is unclear. One explanation may be that the regulation of magnesium flux differs from one cell type to another[39].

Using the polymorphonuclear magnesium content obtained in the control group as reference, we divided the patients with asthma into two groups. Patients who had a polymorphonuclear magnesium content of 0.9 mg Mg/ng DNA or more were included in the subgroup of normal-plMg (n=16), and patients who had a polymorphonuclear magnesium content of less than 0.9 mg Mg/ng DNA were included in the low-plMg content subgroup, the mean plMg content was 0.38 ± 0.28 mg Mg/ng DNA, lower than in the total patient group (P=0.005). In the normal-plMg content subgroup of asthmatics, no variable discriminated between the control group and this subgroup. We think that if the behaviour of intracellular magnesium content were to be studied during and immediately after asthma attacks, intracellular magnesium content might be found to have a role in the normal-plMg content subgroup of patients. The existence of mechanisms that maintain a low intracellular free calcium concentration has been demonstrated, hence, there must also exist mechanisms that increase intracellular calcium concentrations[25]. It may be that the normal-plMg subgroup of patients produce greater mobilization of Ca^{++} in response to the agent triggering the episode of bronchial asthma. Although magnesium intracellular concentration would be normal, higher intracellular calcium concentrations may change the calcium/magnesium ratio, producing a 'relative deficit' of magnesium in relation to calcium concentration. Given that magnesium is a natural calcium antagonist, a relative deficit of magnesium would potentiate the action of calcium. However, these speculations cannot be demonstrated with the findings of our study as our work was carried out on asthmatics between attacks.

Both the low-plMg and normal-plMg subgroups of asthmatics had similar percentages of patients with intrinsic and extrinsic asthma (Figure 1). Thus, there was no

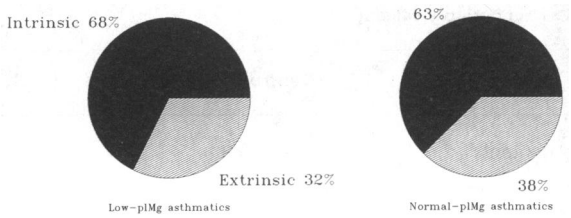

Figure 1 **Percentage of patients with extrinsic and intrinsic asthma in both subgroups of asthmatics. plMg=Polymorphonuclear magnesium content**

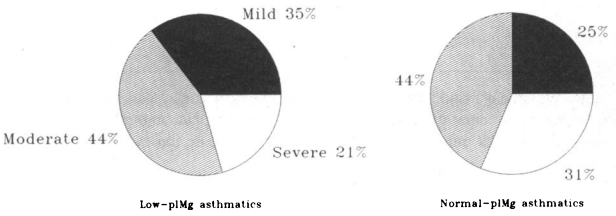

Figure 2 **Percentage of patients with mild, moderate and severe asthma in both subgroups of asthmatics. plMg=Polymorphonuclear magnesium content**

correlation between different types of asthma and intracellular magnesium content. Likewise, in both subgroups of asthmatics, the percentages of patients with mild, moderate and severe asthma were similar (Figure 2). Our study, therefore, did not reveal a correlation between intracellular magnesium content and the degree of asthmatic severity. Medical treatment did not influence polymorphonuclear magnesium content (Figure 2).

Intracellular magnesium may provide relevant information in patients with bronchial asthma. In asthmatics with low intracellular magnesium content, magnesium may improve the condition by normalizing intracellular magnesium content. Appropriate intervention trials may yield valuable information in this interest. In asthmatics with normal intracellular magnesium content, the behaviour of intracellular magnesium content should be studied during and immediately after asthma attacks.

In conclusion, we found that our patients with bronchial asthma in the interval between attacks had a low polymorphonuclear magnesium content, which may contribute to the pathogenesis of asthma. In almost 30% of our asthmatic patients, polymorphonuclear magnesium content was similar to that of the control group and we found no variable that differentiated clearly this subgroup from the control group.

Acknowledgments This work was supported in part by FISS grant number 93/0192.

REFERENCES

1 Okayama H, Takashi A, Okayama M, Sasaki H, Mue S, Takishima T. Bronchodilating effect of intravenous magnesium sulfate in bronchial asthma. *JAMA* 1987;**257**:1076–8

2 McNamara RM, Spivey WH, Skobeloff EM, Jacubowitz S. Intravenous magnesium sulfate in the management of acute respiratory failure complicating asthma. *Ann Emerg Med* 1989;**18**:197–9

3 Rolla G, Bucca C, Caria E, *et al.* Acute effect of intravenous magnesium sulfate on airway obstruction of asthmatic patients. *Ann Allergy* 1986;**61**:388–91

4 Skobeloff EM, Spivey WH, McNamara RM, Greenspon L. Intravenous magnesium sulfate for the treatment of acute asthma in the emergency department. *JAMA* 1989;**262**:1210–13

5 Rolla G, Bucca C, Bugiania M, Arissa W, Spinaci S. Reduction of histamine-induced bronchoconstriction by magnesium in asthmatic subjects. *Allergy* 1987;**42**:186–8

6 Rolla G, Bucca C, Arossa W, Bugiani M. Magnesium attenuates methacholine-induced bronchoconstriction in asthmatics. *Magnesium* 1987;**6**:201–4

7 Engbaek L. The pharmacological actions of magnesium ions with particular reference to the neuromuscular and the cardiovascular system. *Pharmacol Rev* 1952;**4**:396–414

8 Iseri LT, French JH. Magnesium: nature's physiologic calcium blocker. *Am Heart J* 1984;**108**:188–93

9 Levine BS, Coburn JW. Magnesium the mimic/antagonist of calcium. *New Engl J Med* 1984;**310**:1253–55

10 Ishizaka K, Tomioka H, Ishizaka T. Mechanisms of passive sensitization. I. Presence of IgE molecules on human leukocytes. *J Immunol* 1970;**105**:1459–67

11 Ishizaka T, Ishizaka K, Tomioka H. Release of histamine and slow-reacting substance of anaphylaxis (SRS-A) by IgE-anti-IgE reactions on monkey mast cells. *J Immunol* 1972;**108**:513–20

12 Peachell PT, Pearce FI. Calcium regulation of histamine secretion from mast cells. In: Marme D, Ed. *Calcium and Cell Physiology*. New York: Springer, 1985: 311–27

13 Matthew R, Altura BM. Magnesium and the lungs. *Magnesium* 1988;**7**:173–87

14 Hardy CCH, Robinson C, Taatersfield AE, Holgate ST. The bronchoconstrictor effect of inhaled prostaglandin D_2 in normal and asthmatic men. *New Engl J Med* 1984;**311**:209–13

15 Altura BM, Altura BT, Waldemar Y. Prostaglandin-induced relaxations and contractions of arterial smooth muscle: effect of magnesium ions. *Artery* 1976;**2**:1315–17

16 Nigam S, Averdunk R, Gunther T. Alteration of prostanoid metabolism in rats with magnesium deficiency. *Prostaglandins Leuk Med* 1986;**23**:1–10

17 Bois P, Gacon A, Beaulnes A. Histamine liberating effect of magnesium deficit in rats. *Nature* 1963;**197**:501–3

18 Böyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 1968;**21**(suppl 97):77–89

19 English D, Anderson BR. Single-step separation of red blood cells, granulocytes and mononuclear leukocytes in discontinuous density gradient of ficoll-hypaque. *J Immunol Meth* 1974;**5**:249–54

20 Kapuscinski J, Sroczylas B. Simple and rapid fluorometric method for DNA microassay. *Annal Biochem* 1977;**83**:252–7

21 Gaddy JN, Busse WW. Enhanced IgE-dependent basophil histamine release and airway reactivity in asthma. *Am Rev Respir Dis* 1986;**134**:969–74

22 Akagi KA, Townley RG. Spontaneous histamine release and histamine content in normal subjects and subjects with asthma. *J Allergy Clin Immunol* 1989;**83**:742–9

23 Reinhart RA. Magnesium metabolism. A review with special reference to the relationship between intracellular content and serum levels. *Arch Intern Med* 1988;**148**:2415–20

24 Ryzen E, Elkayam U, Rude RK. Low blood mononuclear cell magnesium in intensive cardiac care unit patients. *Am Heart J* 1986;**111**:475–80

25 Middleton E Jr. Antiasthmatic drug therapy and calcium ions. Review of pathogenesis and role of calcium. *J Pharm Sci* 1980;**69**:243–51

26 Bierman CW, Pearlman DS. Asthma. In: Cherninck V, Kending EL, Eds. *Disorders Of the Respiratory Tract In Children*. Philadelphia: W B Saunders Company, 1990: 557–601

27 Altura BM, Altura BT. New perspectives on the role of magnesium in pathophysiology of the cardiovascular system II. Experimental aspect. *Magnesium* 1985;**4**:245–71

28 Parker CW, Smith JW. Alterations in cyclic adenosine monophosphate metabolism in human bronchial asthma. *J Clin Invest* 1973;**52**:48–59

29 Schulman ES, Newball HH, Demers LM, Fitzpatrick FA, Adkinson NF Jr. Anaphylactic release of thromboxane A2, prostaglandin D_2 and prostacyclin from human lung parenchyma. *Am Rev Resp Dis* 1981;**124**:402–6

30 Altura BM, Altura BT. Vascular smooth muscle and prostaglandins. *Fed Proc* 1976;**35**:2360–6

31 Hui KP, Barnes NC. Inhibition of leukotriene synthesis: new therapy in asthma? *Pulm Pharmacol* 1993;**6**:3–9

32 Wagner CD, Gundel RH, Abraham WM, *et al.* The role of 5-lipoxygenase products in preclinical models of asthma. *J Allergy Clin Immunol* 1993;**91**:917–29

33 Pacheco Y, Hosni R, Chabannes B, *et al.* Leukotriene B4 level in stimulated blood neutrophils and alveolar macrophages from healthy and asthmatic subjects. Effect of beta-2 agonist therapy. *Eur J Clin Invest* 1992;**22**:732–9

34 Abraham WM, Ahmed A, Cortes A. The 5-lipoxygenase inhibitor zileuton blocks antigen-induced late airway hyperresponsiveness in allergic sheep. *Eur J Pharmacol* 1992;**217**:119–26

35 Samuelsson B, Dahlen SE, Lindgreen J, Rouzer CA, Serhan CN. Leukotrienes and lipoxins: structure, biosynthesis and biological effects. *Science* 1987;**237**:1171–5

36 Ulevitch RJ, Watanabe Y, Sano M, Lister MD, Deems RA, Dennis EA. Solubilization, purification, and characterization of a membrane-bound phospholipase A_2 from the $P388D_1$ macrophage-like cell line. *J Biol Chem* 1988;**263**:3079–85

37 Rouzer CA, Samuelsson B. Reversible, calcium-dependent membrane association of human leukocyte 5-lipoxygenase. *Proc Natl Acad Sci USA* 1987;**84**:7393–7

38 Aderem AA, Cohn ZA. Calcium ionophore synergizes with bacterial lipoplysaccharides in activating macrophage arachidonic acid metabolism. *J Expl Med* 1988;**167**:623–31

39 Maguire ME. Hormonal regulation of magnesium uptake: Differential coupling of membrane receptors to magnesium uptake. *Magnesium* 1987;**6**:180–91

(Accepted 11 November 1994)